FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)
01017/36667	10/049182

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/23110	23 August 2000	23 August 1999

TITLE OF INVENTION
MODULATION OF THE BLOOD-BRAIN BARRIER TRANSPORTER FOR LEPTIN

APPLICANT(S) FOR DO/EO/US
BANKS, WILLIAM A.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Sequence Statement Pursuant to 35 U.S.C. 1.821-1.825; Return receipt postcard.

JC10 Rec'd PCT/PTO 08 FEB 2002

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/049182	PCT/US00/23110	01017/36667

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1040.00
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$890.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$740.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$710.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	$0.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	76 - 20 =	56	x $18.00	$1,008.00	
Independent claims	22 - 3 =	19	x $84.00	$1,596.00	
Multiple Dependent Claims **(check if applicable).**			☒	$280.00	
TOTAL OF ABOVE CALCULATIONS =				$3,774.00	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				$0.00	
SUBTOTAL =				$3,774.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				$0.00	
TOTAL NATIONAL FEE =				$3,774.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				$0.00	
TOTAL FEES ENCLOSED =				$3,774.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$3,774.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **13-2855** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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NAME

43,363

REGISTRATION NUMBER

08 February 2002

DATE

MODULATION OF THE BLOOD-BRAIN BARRIER TRANSPORTER FOR LEPTIN

RELATED APPLICATIONS

This application claims priority from U.S. Provisional Patent Application Serial No. 60/150,300, filed August 23, 1999.

FIELD OF THE INVENTION

The present invention relates generally to modulating the body weight and/or appetite of mammals, including humans. More particularly this invention relates to compositions and methods which modulate the transport of weight-controlling molecules, such as leptin, across the blood-brain barrier.

GOVERNMENT RIGHTS

This invention was made with support from the United States Government. The United States Government retains certain rights to this invention.

BACKGROUND OF THE INVENTION

Obesity is defined as an excess of body fat relative to lean body mass and is associated with important psychological and medical morbidities, including hypertension, elevated blood lipids, and diabetes. Body weight and energy balance are thought to be regulated by a feedback mechanism in which the regions of the brain, for example, the hypothalamus, senses the amount of energy stored in the body then adjusts food intake and activity level accordingly [Brobeck, J.R., *Yale J. Biol. Med,* **20**:545-552 (1948)]. Early experiments showed that the arterial transfer of blood from one animal having a hypothalamic lesion to a normal healthy animal resulted in the reduction of food intake by the normal animal [Hervey, G. H., *J. Physiol.*, **145**:336-352 (1959)]. From these results it was hypothesized that at least one component of the feedback mechanism circulated through the bloodstream and that the component acted on the brain. It has been suggested that the OB gene may be

responsible for the generation of this blood-borne factor [see also Coleman, D.L., *Diabetologica*, **14**:141-148 (1978)].

Recent studies of the OB gene have confirmed that the OB gene product known as leptin is the blood-borne factor which works to maintain body weight and energy balance [Zhang *et* al., *Nature.*, **372**:425-432 (1994); and Freidman *et al.*, PCT Application No. PCT/US95/10479]. Further, it has also been shown that the administration of leptin results in a decreased amount of body fat [Pelleymounter, M.A. *et al. Science*, **269**:540-543 (1995); Halaas, *et al. Science*, **269**:543-546 (1995); Campfield, *et al. Science*, **269**:546-549 (1995)]. It is believed that leptin acts on the brain to inhibit food intake, regulate energy expenditure, and control body weight.

In order for leptin to play this type of role, leptin must cross over the blood-brain barrier to enter the brain. The amount of leptin sensed by the brain results from a combination of the permeability of the blood brain barrier and the amount of leptin in the bloodstream which in turn depends on the level of stored energy or body fat of an individual [Considine, R. V. *et al., N. Eng. J. Med.* **334**:292-295 (1986)]. Obesity can occur when the brain incorrectly senses a low level of leptin and so initiates mechanisms to raise that level by increasing the amount of body fat. This cycle usually continues until the brain senses an appropriate amount of leptin at which time the body weight ceases to increase. As described herein, it is believed that increasing the efficiency of leptin transport across the blood-brain barrier would be an effective treatment for obesity, in most cases.

Blood-borne leptin is able to enter the brain because of the presence of a specific saturable transporter located at the blood-brain barrier [Banks *et al.*, *Peptides* **17(2)**:305-311 (1996)]. Because leptin is a large protein, leptin in the blood would be largely excluded from the brain in the absence of such a transporter. It is believed that the transporter is close to or contains within its structure some sites which, when activated, modify the transport rate of leptin. Such sites, conceptually analogous to cofactors binding sites for enzymes or allosteric regulatory sites for receptors, provide therapeutic targets which can be manipulated to alter the rate of leptin transport from the blood into the brain so as to control body weight.

The mechanism of transport of proteins and peptides such as leptin across the blood-brain barrier is poorly understood. Some proteins/peptides cross this barrier by diffusing directly through the endothelial/ependymal membranes according to their lipophilicity and/or molecular weight with smaller lipophilic molecules passing more freely [Banks & Kastin, *Psychoneuroendocrinology,* **10**:385-399 (1985)]. Others are transported by saturable, carrier-mediated systems [Banks & Kastin, *Pharmacol. Biochem. Behav.,* **21**:943-946 (1984)], such as the one that transports Tyr-MIF (Tyr-Pro-Leu-Gly-amide) [Banks & Kastin, *J. Pharmacol. Exp. Ther,* **239**:668-672 (1986)]. Another mechanism by which proteins or polypeptides cross the blood-brain barrier is through receptor-mediated permeabilization which is enabled by the administration of molecules such as bradykinin, leukotrienes, histamine, and 5-hydroxytryptamine [Unterberg, A., *J. Cereb. Blood Flow Metab.,* **4**:574-585 (1984)]. Similarly, molecules such as leucine encephalin, α-adrenergics, arachidonic acid, aluminum, phorbomyristate esters, and α-thrombin increase blood-brain barrier permeability while angiotensin II and β-adrenergics reduce the permeability [Grieg, N., *Physiology and Pharmacology of the Blood-Brain Barrier Handbook of Experimental Pharmacology,* **103**:487-523 Springer-Verlag, Berlin (1992)].

There is a considerable need for molecules or compositions and methods for using those molecules and compositions which modulate (i.e., enhance or inhibit) the transport of weight-controlling molecules, such as leptin, across the blood-brain barrier.

SUMMARY OF THE INVENTION

The present invention is directed to methods and compositions for modulating feeding behavior and/or appetite in mammals as well as for modulating body weight in mammals. More particularly, the present invention is directed to the methods and compositions for modulating (enhancing or inhibiting) the transport of leptin across the blood-brain barrier and across other blood/tissue barriers. The invention is also directed to methods and compositions for modulating (increasing or decreasing) body weight and/or metabolism by altering the transport of leptin across

the blood-brain barrier. According to the invention, leptin transport across the blood-brain barrier may be increased, thereby resulting in a reduction in body weight, and/or a decrease in appetite. Conversely, leptin transport across the blood-brain barrier may be decreased (or inhibited) resulting in an increase in appetite and/or body weight in patients in need thereof (e.g., anorexia, cachexia of aging, tumor-induced cachexia). The compositions may act on either side of the blood-brain barrier (or other blood-tissue barriers) to result in altered transport of leptin, although preferred compositions and methods act on the blood side of the barrier.

A preferred method of the invention comprises administering to a subject in need thereof a composition which comprises an adrenergic agonist in an amount effective to increase the transport of leptin or leptin variants, analogs, fragments, consensus leptin, or derivatives (including but not limited to a fusion protein) or chemically modified derivatives of leptin across the blood-brain barrier. A fusion protein refers to a protein comprising a leptin polypeptide and a different protein. The methods of the invention allow the enhancement of the transport of either endogenous leptin or exogenous leptin (including analogs, fragments, consensus leptin, chemical derivatives thereof or fusion protein) across the blood-brain barrier. Conversely, in another embodiment of the invention, an adrenergic antagonist may be used to inhibit leptin transport across the blood-brain barrier. An exemplary adrenergic antagonist which acts to inhibit leptin transport into the brain thereby resulting in an increase in body weight includes but is not limited to benoxathian, may be administered to an individual to increase body weight. According to the present invention both purinergic and glutaminergic agonists may also be used to modulate leptin transport into the brain. An exemplary purinergic agonist comprises adenosine while an exemplary glutaminergic agonist comprises glutamate.

Routes of administration of the compositions useful in the practice of the invention include but are not limited to intravenous, intraarterial, intraperitoneal, intramuscular, intradermal, topical, intraocular, subcutaneous, intranasal, oral, intracisternal, intracerebroventricular, intrathecal, topical, intradermal, or pulmonary.

Conversely, in another preferred embodiment, an adrenergic antagonist may be used to inhibit leptin transport across the blood-brain barrier.

In one embodiment of the present invention, the composition comprises one or more compounds selected from the group consisting of adrenergic agonists such as, but not limited to, epinephrine, isoproterenol, arterenol, cirazoline, phenylethylamine, epinephrine, norepinephrine, dopamine, nordefrin, protokylol, metaproterenol, metaraminol, phenylehprine, tyramine, hydroxyamphetamine, nylidrin, isoxsuprine, methoxyphenamine, methoxamine, amphetamine, methamphetamine, ephedrine, phenylpropanolamine, mephentermine, chlorphentermine, tuaminoheptane, cyclopentamine, propylhexedrine, and analogs and derivatives or metabolites thereof and optionally, a pharmaceutically acceptable carrier, excipient or diluent. Exemplary adrenergic antagonists includes, but are not limited to, phentolamine, prazosin, benoxathian, phenotybenzamine, and related laloallyl-aminos.

In another embodiment, the compositions of the invention comprise purinergic and glutaminergic agonists or combinations thereof and their use in the methods of the invention. Adenosine activates the adenosine, or purinergic 1 (P1) receptor. P1 receptors are widespread in the body including the cardiovascular, respiratory, immune, and nervous systems. Adenosine blocks opioid-induced feeding and caffeine is a P1 antagonist.

Glutamate is the endogenous ligand for glutamate (glutaminergic) receptors. Glutamate receptors include ionotropic receptors (AMPA, kainate, and N-methyl-D-aspartate receptors), which directly control ion channels, and metabotropic receptors which act through second messenger systems. Glutamate receptors are the most common mediators of fast excitatory synaptic transmission in the central nervous system. They are implicated in the mechanisms of memory and feeding.

Other compounds that affect feeding, suppress appetite, induce anorexia, stimulate appetite, affect weight, or alter metabolism and which may ultimately affect leptin transport across the blood-brain barrier and which are useful in the practice of the present invention include free fatty acids, sugars such as glucose, cytokines, drugs such as amphetamines, calcium channel blockers, monoamines,

amino acids, hormones including steroid hormones, dietary supplements, ketones, starches, micronutrients, lipoproteins, prostaglandins, prostacyclins, peptides, proteins, regulators of nitric oxide production, NMDA and GABA agonists and antagonists, vitamins, minerals, and melatonin, and their precursors and metabolites.

Preferred cytokines useful in the practice of the present invention include, but are not limited to, interleukin 1α, interleukin 1β, interleukin 1 receptor antagonist, interleukin 2, interleukin 6, interleukin 12, macrophage colony stimulating factor, macrophage inflammatory peptides such as MIP-1α, MIP-1β, and tumor necrosis factor α (TNFα).

Other compounds used in the practice of the present invention include fenflurimine and related compounds.

Other peptides and proteins useful in the practice of the present invention either alone or in combination with other compounds described herein include, but are not limited to, adrenocorticotropin hormone (ACTH), amylin, atrial natriuretic peptide (ANP), bombesin, calcitonin, calcitonin gene related peptide (CGRP), caerulein, cocaine and amphetamine regulated transcript peptide (CART), cholecystkinins (CCK), corticotropin releasing hormone (CRH), Cyclo-His-Pro, enterostatin, FMRF-amide, galanin, glucagon, glucagon-like peptide (GLP), growth hormone, growth hormone releasing hormone (GHRH), gonadotropin hormone releasing hormone (GnRH or LHRH), insulin, insulin-like growth factors, macrophage migration inhibiting factor, melanocyte stimulating hormone (MSH), motilin, MSH-inhibitory peptide (MIF-1), nerve growth factor (NGF), neuromedins, neuropeptide Y (NPY), neurotensin, neurotrophins (NT-3, NT-4), opiate peptides (endorphins, enkephalins, endomorphins, dynoorphins, kyotorphin), orexin, oxytocin, pancreatic polypeptide, parathyroid hormone (PTH), pituitary adenylate cyclase activating polypeptides (PACAP), sauvagine, somatostatin, substance P, thyroid stimulating hormone (TSH), thyrotropin releasing hormone (TRH), tyrosine MIF-1, vasoactive intestinal polypeptide, and vasopressins.

Other compositions used in the practice of the present invention comprise any of the foregoing compositions in combination with one another and/or in combination with one or more of the leptins described herein.

This invention is also directed to a method for treating obesity which comprises enhancing the transport of leptin, leptin variants, analogs, consensus leptins, fragments, or leptin derivatives thereof across the blood-brain barrier according to any of the preceding aspects or embodiments.

In yet another embodiment of this invention, methods and compositions for treating metabolic disorders including obesity, diabetes mellitus, including type I and type II diabetes and insulin-resistant pathologies which comprise enhancing the transport of leptin, leptin variants, analogs, consensus leptins, fragments, or derivatives thereof across the blood-brain barrier according to any of the preceding aspects or embodiments are provided.

Also within the scope of the present invention are pharmaceutical compositions useful for modulating body weight, the composition comprising leptin comprising the amino acid sequence set out in SEQ ID NO: 2 or 4, SEQ ID NO: 5 and SEQ ID NO: 6, consensus leptins, variants, analogs, leptin fusion proteins, chemically modified derivatives of leptin, and fragments thereof, and one or more agents selected from the group consisting of adrenergic agonists, adrenergic antagonists, neurotransmitters, peptide hormones, cytokines, amino acids, opiate peptides, purinergic agonists, purinergic antagonists, glutaminergic agonists and glutaminergic antagonists, and metabolites thereof.

The invention also includes compositions and methods for modulating body weight and/or treating metabolic disorders by modulating the regulatory pathways which control appetite and/or metabolism. Because leptin appears to play a controlling role in appetite regulation, the methods and compositions of the invention are useful for modulating regulatory pathways in which the leptin plays a role, perhaps ultimately by regulating the transport of leptin across the blood-brain barrier.

The invention also comprises the use of adrenergic agonists, adrenergic antagonists, neurotransmitters, peptide hormones, cytokines, amino acids, opiate peptides, purinergic agonists or antagonists, glutaminergic agonists or antagonists, or metabolites thereof for the manufacture of a medicament for modulating leptin transport into the brain and/or for modulating body weight and/or for modulating appetite in a mammal. The uses may further comprise the use of any of the leptins

within the scope of the invention for the manufacture of the medicament for modulating the transport of leptin across the blood-brain barrier and/or for modulating the body weight of a mammal. Preferred mammals for the practice of the present invention are humans.

DETAILED DESCRIPTION OF THE INVENTION

The mammalian brain plays a central role in regulating the amount of fat in a mammal in part by regulating food intake, food selection, and thermogenesis. The brain senses the fat level (adiposity) of the organism by sensing the amount of leptin in the blood of the organism which is transported into the brain via a specific saturable leptin transporter located at the blood brain barrier. Obesity can occur when the brain incorrectly senses less than the appropriate amount of leptin in the organism which thereby triggers mechanisms to increase adiposity (e.g., increasing feeding, decreasing metabolic rate). Adiposity then increases until the brain senses an appropriate level of leptin. It, therefore, follows that increasing the efficiency of transporting leptin across the blood brain barrier would be an effective way to reduce adiposity by increasing the amount of leptin effectively sensed by the brain.

Evidence suggests that the transporter responsible for leptin transport across the blood-brain barrier is associated with or contains within its structure sites that when actuated modify the rate of leptin transport. These transporter rate modifying sites are conceptually analogous to co-factor and/or allesteric regulatory sites for enzymes or co-factors. The presence of such sites therefore provide attractive therapeutic targets that can be used to regulate the transport of leptin across the blood-brain barrier thereby regulating adiposity in the mammal.

The present invention provides compositions and methods for modulating body weight by modulating the signaling pathways involved in weight regulation and/or appetite regulation. The invention also provides compositions and methods for modulating the transport of leptin across the blood-brain barrier and materials and methods for modulating appetite.

More particularly, the present invention is directed to compositions including pharmaceutical compositions and methods for enhancing or inhibiting the

transport of leptin (OB) polypeptides across the blood-brain barrier. Such methods and compositions are useful in controlling the body weight of mammals, including humans. The methods and compositions are also useful in the treatment of metabolic disorders including diabetes mellitus (type I and type II). The compositions and methods of the present invention exploit the central role of leptin in the regulation of appetite and metabolism by modulating the transport of leptin across the blood-brain barrier to a site of action in the brain.

For the purposes of this invention, leptin and OB are used interchangeably and refer to a polypeptide having as a mature form about 146 amino acids.

Any leptin molecule, including leptin variants, analogs, fragments, consensus leptins, or derivatives, which have the ability to modulate weight, or to alter metabolism in a host mammal, is useful in the practice of the present invention. Preferred leptin proteins useful in the practice of the present invention may be native murine leptin set out as SEQ ID NO: 2 which includes its signal sequence, or its mature form beginning at amino acid 21 (as numbered in SEQ ID NO: 2) of native leptin and set out as SEQ ID NO: 5 or protein as set forth in Zhang *et al.* (*Nature*, *supra*, herein incorporated by reference) or the native human OB protein (SEQ ID NO: 4) or its mature sequence beginning amino acids 21 through 166 set out as SEQ ID NO: 6. (*See* Zhang *et al.*, Nature *supra*, at page 428.) Variants or analogs of the leptin proteins useful in the practice of the present invention include those having a substitution of one or more of its amino acids with another while still maintaining a biological activity of leptin. Natural variants of either leptin which lack a glutamine residue at position 28 of the mature sequence or other natural variants are also useful in the practice of the invention. Another example of a human leptin useful in the practice of the invention is an analog of SEQ ID NO: 6, which comprises 1) an arginine in place of lysine at position 35; and 2) a leucine in place of isoleucine at position 74. (A shorthand abbreviation for this analog is the recombinant human R->K^{35}, L->I^{74}). The leptin molecules useful in the practice of the present invention may also optionally comprise a methionine at the N-terminus (-1 position).

The murine leptin protein has significant homology to the human protein, particularly as a mature protein, and, further, particularly at the N-terminus. One may prepare an analog of the recombinant human protein for use in the practice of the present invention by altering (such as substituting amino acid residues), in the recombinant human sequence, the amino acids which diverge from the murine sequence. For example, using a human protein having a lysine at residue 35 and an isoleucine at residue 74 according to the numbering of SEQ ID NO: 6, wherein the first amino acid is valine, and the amino acid at position 146 is cysteine, one may substitute with another amino acid one or more of the amino acids at positions 32, 35, 50, 64, 68, 71, 74, 77, 89, 97, 100, 101, 105, 106, 107, 108, 111, 118, 136, 138, 142, and 145. One may select the amino acid at the corresponding position of the murine protein, (SEQ ID NO: 6), or another amino acid.

One may further prepare "consensus" molecules (consensus leptin or consensus OB) based on the rat OB protein sequence [Murakami *et al.*, *Biochem. Biophys. Res. Comm.* **209**:944-952 (1995) herein incorporated by reference]. Rat OB protein differs from human OB protein at the following positions (using the numbering of SEQ ID NO: 6): 4, **32**, 33, **35**, **50**, 68, **71**, **74**, **77**, 78, **89**, **97**, **100**, **101**, 102, **105**, **106**, **107**, **108**, **111**, **118**, **136**, **138**, and **145**. One may substitute with another amino acid one or more of the amino acids at these divergent positions. The positions underlined and in bold print are those in which the murine leptin protein as well as the rat OB protein are divergent from the human OB protein, and thus, are particularly suitable for alteration. At one or more of these positions, one may substitute an amino acid from the corresponding rat OB protein, or another amino acid.

The positions from both mature rat and mature murine OB protein which diverge from the mature human leptin protein, are: 4, 32, 33, 35, 50, 64, 68, 71, 74, 77, 78, 89, 97, 100, 101, 102, 105, 106, 107, 108, 111, 118, 136, 138, 142, and 145. A human OB protein according to SEQ ID NO: 6 having one or more of the above amino acids deleted or replaced with another amino acid such as the amino acid found in the corresponding rat or murine sequence may also be effective.

In addition, the amino acids found in rhesus monkey leptin protein which diverge from the mature human OB protein are (with identities noted in parentheses in one letter amino acid abbreviation): 8(S), 35(R), 48(V), 53(Q), 60(I), 66(I), 67(N), 68(L), 89(L), 100(L), 108(E), 112(D), and 118(L). Since the recombinant human OB protein is active in cynomolgus monkeys, a human OB protein according to SEQ ID NO: 4 or 6 having one or more of the rhesus monkey divergent amino acids replaced with another amino acid, such as the amino acids in parentheses, may be effective. It should be noted that certain rhesus divergent amino acids are also those found in the above murine species (positions 35, 68, 89, 108, and 118). Thus, one may prepare a murine/rat/rhesus/human consensus molecule (using the numbering of SEQ ID NO: 6 having one or more of the amino acids at positions replaced by another amino acid: 4, 8, 32, 33, **35**, 48, 50, 53, 60, 64, 66, 67, **68**, 71, 74, 77, 78, **89**, 97, **100**, 101, 102, 105, 106, 107, **108**, 111, 112, 118, 136, 138, 142, and 145. The positions underlined and in bold print are those in which all three species are divergent from the human OB protein, and thus, are particularly suitable for alteration.

Other analogs may be prepared by deleting a part of the protein amino acid sequence which results in a fragment of a leptin polypeptide. For example, the mature protein lacks a leader sequence which corresponds to amino acids 1-21 of SEQ ID NO: 4. One may prepare the following truncated forms of the native human leptin protein molecules (using the number of SEQ ID NO: 6):

(a) amino acids 98-146

(b) amino acids 1-32

(c) amino acids 40-116

(d) amino acids 1-99 and (connected to) 112-146

(e) amino acids 1-99 and (connected to) 112-146 having one or more of amino acids 100-111 placed between amino acids 99 and 112.

Also, the truncated forms (fragments) may also have altered one or more of the amino acids which are divergent (in the rhesus, rat or murine OB protein) from human OB protein. Furthermore, any alterations may be in the form of altered amino acids, such as peptidomimetics or D-amino acids. Further, leptin molecules

having 83% or more amino acid identity with leptins having the amino acid sequence set out in SEQ ID NOs: 2, 4, 5 or 6 may also be used in the practice of the invention.

Any of the foregoing leptin molecules may optionally have an N-terminal methionine.

Also included with the scope of the invention are leptins encoded by any of the polynucleotides set out in U.S. Patent No. 5,935,810 or any of the polypeptides set out in U.S. Patent No. 6,001,968 which are incorporated by reference in their entirety.

The present protein (herein the term "protein" is used to include "peptide" and OB analogs, such as those recited above, unless otherwise indicated) may also be derivatized by the attachment of one or more chemical moieties to the protein moiety. The chemically modified derivatives may be further formulated for intraarterial, intraperitoneal, intramuscular, subcutaneous, intravenous, oral, nasal, pulmonary, topical, ocular, intracisternal, intrathecal, transdermal, intracerebroventricular, or other routes of administration. Chemical modification of biologically active proteins has been found to provide additional advantages under certain circumstances, such as increasing the stability and circulation time of the therapeutic protein and decreasing immunogenicity. *See* U.S. Patent No. 4,179,337, Davis *et al.*, issued December 18, 1979. For a review, *see* Abuchowski *et al.*, *Enzymes as Drugs* (J.S. Holcenberg and J. Roberts, eds. pp. 367-383 (1981)). A review article describing protein modification and fusion proteins is Francis, *Focus on Growth Factors* 3:4-10 (May 1992) (published by Mediscript, Mountview Court , Friern Barnet Lane, London N20, OLD, UK).

The chemical moieties suitable for derivatization may be selected from among various water soluble polymers. The polymer selected should be water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable. One skilled in the art will be able to select the desired polymer based on such considerations as whether the polymer/protein conjugate will be used therapeutically, and if so, the desired dosage, circulation time, resistance to proteolysis, and other

considerations. For the present proteins and peptides, the effectiveness of the derivatization may be ascertained by administering the derivative, in the desired form (i.e., by osmotic pump, or, more preferably, by injection or infusion, or, further formulated for oral, pulmonary or nasal delivery, for example), and observing biological effects as described herein.

The water soluble polymer may be selected from the group consisting of, for example, polyethylene glycol, copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrolidone, poly-1, 3-dioxolane, poly-1, 3, 6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random or non-random copolymers), and dextran or poly (n-vinyl pyrolidone) polyethylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols, polystyrenemaleate and polyvinyl alcohol. Polyethylene glycol propionaldenhyde may have advantages in manufacturing due to its stability in water.

Fusion proteins may be prepared by attaching polyaminoacids to the OB protein (or analog) moiety. For example, the polyaminoacid may be a carrier protein which serves to increase the circulatory half-life of the protein. For the present therapeutic or cosmetic purposes, such polyaminoacid should be those which do not create neutralizing antibody response, or other adverse response. Such polyaminoacid may be selected from the group consisting of serum albumin (such as human serum albumin), an antibody or portion thereof (such as an antibody constant region, sometimes called "F_c") or other polyaminoacids. As indicated below, the location of attachment of the polyamino acid may be at the N-terminus of the OB protein moiety, or other place, and also may be connected by a chemical "linker" moiety to the OB protein.

In the case of an OB-Fc fusion, the OB is typically fused at its C-terminus with the N-terminus. However, OB may be fused at its N-terminus with the C-terminus of the Fc molecule. Typically, in such fusions, the fused protein will retain at least functionally active hinge CH2 and CH3 domains of the constant region of the immunoglobulin heavy chain. Fusions may also be made to the C-terminus of the Fc portion of a constant domain or immediately N-terminal to the CH1 domain of

the heavy chain or the corresponding region of the light chain. The exact site at which the fusion is made is not critical. The fusion proteins may comprise multimers of the Fc-OB fusion.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 2 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The number of polymer molecules so attached may vary, and one skilled in the art will be able to ascertain the effect on function. One may mono-derivatize, or may provide for a di-, tri-, tetra- or some combination of derivatization, with the same or different chemical moieties (e.g., polymers, such as different weights of polyethylene glycols). The proportion of polymer molecules to protein (or peptide) molecules will vary, as will their concentrations in the reaction mixture. In general, the optimum ratio (in terms of efficiency of reaction in that there is no excess unreacted protein or polymer) will be determined by factors such as the desired degree of derivatization (e.g., mono, di-, tri-, etc.), the molecular weight of the polymer selected, whether the polymer is branched or unbranched, and the reaction conditions.

The chemical moieties should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art. *E.g.*, EP 0 401 384 herein incorporated by reference (coupling PEG to G-CSF), *see also* Malik , *Exp. Hematol.* **20**:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and

the N-terminal amino acid residue. Those having a free carboxyl group may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Sulfhydryl groups may also be used as reactive groups for attaching the polyethylene glycol molecule(s). Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group. Attachment at residues important for receptor binding should be avoided if receptor binding is desired.

One may specifically desire N-terminally chemically modified OB protein or polypeptides. Using polyethylene glycol as an illustration of the compositions useful in the practice of the present invention, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective N-terminal chemical modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved. For example, one may selectively N-terminally pegylate the protein by performing the reaction at a pH which allows one to take advantage of the pK_a differences between the ϵ-amino group of the lysine residues and that of the α-amino group of the N-terminal residue of the protein. By such selective derivatization, attachment of a water soluble polymer to a protein is controlled: the conjugation with the polymer takes place predominantly at the N-terminus of the protein and no significant modification of other reactive groups, such as the lysine side chain amino groups, occurs. Using reductive alkylation, the water soluble polymer may be of the type described above, and should have a single reactive aldehyde for coupling to the protein. Polyethylene glycol propionaldehyde, containing a single reactive aldehyde, may be used.

An N-terminally monopegylated derivative is preferred for ease in production of a therapeutic, N-terminal pegylation ensures a homogenous product as characterization of the product is simplified relative to di-, tri- or other multi-pegylated products. The use of the above reductive alkylation process for preparation of an N-terminal product is preferred for ease in commercial manufacturing.

As described in the following examples, the administration of compositions which interact with an adrenoreceptor (preferably adrenergic agonists) either prior to or concurrently with the administration of leptin significantly increases the amount of leptin which crosses the blood-brain barrier into the brain. The compositions and methods of the invention are also useful for increasing the transport of endogenous leptin across the blood-brain barrier. These results are illustrated by the following Examples in which radiolabelled leptin was administered then measured in mice who were given compositions which interact with an adrenoreceptor. Compositions containing epinephrine (which reacts with an adrenoreceptor) were the most effective in enhancing leptin transport. Other compositions including those containing amino acids or hormones were tested as well and in some cases were shown to be effective in enhancing leptin transport across the blood-brain barrier.

The invention is described in the following examples by way of illustration and should not be construed as limiting the invention as set out in the appended claims.

Example 1 describes the effects of epinephrine on leptin transport across the blood-brain barrier.

Example 2 describes the effect of various dosages of epinephrine on the transport of leptin across the blood-brain barrier.

In Example 3, the effect of epinephrine on the integrity of the blood-brain barrier was examined.

In Example 4, the effect of the amino acids tyrosine and phenylalanine on transport of leptin across the blood-brain barrier was studied.

In Example 5, the effect of arginine, phenylalanine, tryptophan, and tyrosine on the transport of leptin across the blood-brain barrier was studied.

In Example 6, the effects of neurotransmitters including dopamine, histamine, serotonin, and epinephrine on leptin transport are described.

Example 7 describes the effect of co-administration of the adrenoreceptor agonists/antagonists cirazoline hydrochloride, UK14304, albuterol, CGP-12177A, and benoxathian hydrochloride on transport of leptin across the blood-brain barrier was examined.

In Example 8, the effect of co-administration of certain adrenoreceptor agonists such as isoproterenol, clonidine, arterenol, and phenylephrine on transport of leptin across the blood-brain barrier was examined.

In Example 9, the effect of the adrenoreceptor antagonists phentolamine, D,L-propanolol, yohimbine, and prasozin on transport of leptin across the blood-brain barrier was tested.

Example 10 describes the effect of tumor necrosis factor on leptin transport across the blood-brain barrier.

Example 11 describes the effect of purinergic and glutaminergic agonists on the transport of leptin across the blood-brain barrier.

EXAMPLE 1

Effects of Administration of Epinephrine on Transport of Leptin across the Blood-Brain Barrier in Mice

In this Example, the effect of administering epinephrine on the transport of leptin across the blood-brain barrier in mice was studied.

In these experiments, six groups of five male ICR mice (Blue Spruce Farms, Altamont, NY) weighing about 17-22 g were anaesthetized with ethyl carbamate (4 g/kg) then had their jugular vein and carotid artery surgically exposed. The mice were then given an intraperitoneal (i.p.) injection of epinephrine (33 µg/200 µl) in lactated Ringer's solution with 1% bovine serum albumin. The time of these injections was considered time zero. After time intervals of 10 minutes (min.), 30 min., 45 min., 1 hour (h), and 2 h post epinephrine injection, radiolabelled leptin (^{125}I, 1.65 x 10^6 cpm) in lactated Ringer's solution with 1% bovine serum albumin was administered to the mice via intravenous (i.v.) injection in the jugular vein. The control mice were not given epinephrine, only lactated Ringer's solution with 1%

bovine serum albumin and were injected with ^{125}I- leptin only after the time interval of 10 minutes. All mice were decapitated and their blood collected after 10 minutes following the leptin injection. The brain (except pituitary and pineals) was removed and counted in a gamma counter (Micromedic 4/200, Horsham, PA) for 3 minutes. Blood was collected from a cut in the right carotid artery, centrifuged at 2000 g for 10 min. at 4°C, then 0.1 ml was counted in a gamma counter. Brain/blood ratios were expressed as counts algebraically to µl/g of brain over counts/min./µl of arterial blood.

Table 1. Administration of Epinephrine Followed by Leptin

Time post epinephrine administration	^{125}I-leptin, brain/serum ± std. dev. (cpm/g)/(cpm/µl)
control (10 min., no epinephrine)	15.68 ± 2.28
10 min.	23.69 ± 3.90
30 min.	23.27 ± 3.45
45 min.	18.68 ± 2.19
1 h	20.73 ± 2.68
2 h	24.86 ± 4.02

The results of this experiment indicate that the administration of epinephrine prior to the administration of leptin enhances the uptake of leptin by the brain in mice. More specifically, in the control mice who received no epinephrine, the amount of radiolabelled leptin in the brain following its i.v. administration was 15.68±2.28 counts/min./g of brain over counts/min./µl of arterial blood as compared to the amount of leptin in the mice who received epinephrine prior to leptin administration was 23.69±3.90 counts/min./g of brain over counts/min./µl of arterial blood. This represents an enhancement in leptin uptake by the brain of approximately 51%. The other time points of 30 min., 45 min., 1 h, and 2 h illustrate that epinephrine still exerts its positive effects on leptin uptake by the brain even after a time interval of 2 h has passed.

EXAMPLE 2

Effects of Administration of Epinephrine on Blood-Brain Barrier in Mice

In this Example, the effect of various dosages of epinephrine on the transport of leptin across the blood-brain barrier was studied.

As in Example 1, mice were anaesthetized with ethyl carbamate. The mice were then given an i.v. injection of a solution containing radiolabelled leptin (^{125}I, 2.1 x 10^6 cpm) in lactated Ringer's solution with 1% bovine serum albumin and various amounts of epinephrine (133.33 µg, 400 nM; 66.6 µg, 200 nM; 33.3 µg, 100 nM; 13.3 µg, 40 nM; 0.667 µg, 2 nM) in 200 µl. Blood and brain samples were collected as described in the previous Examples at 10 min post leptin injection.

Table 2. Co-administration of Epinephrine with Leptin

Time post epinephrine administration (10 min.)	^{125}I, brain/blood ± std. dev. (cpm/g)/(cpm/µl)
control (no epinephrine)	24.54 ± 5.6
+ epinephrine 2 nM	28.68 ± 10.7
+ epinephrine 40 nM	62.73 ± 27.9
+ epinephrine 100 nM	74.18 ± 20.3
+ epinephrine 200 nM	64.75 ± 21.3
+ epinephrine 400 nM	all mice died instantly

The results of this experiment indicate that the co-administration of the leptin plus epinephrine enhances the uptake of leptin by the brain. Likewise, at 40 nM epinephrine the uptake was increased by about 155%, at 100 nM epinephrine the uptake increased by about 200%, at 200 nM epinephrine the uptake was increased about 163% but two of the five mice in that group died, and at 400 nM epinephrine all of the mice died. These data were corrected for the amount of residual blood in the brain after removal of capillaries by gradient centrifugation.

EXAMPLE 3

Effects of Administration of Epinephrine on the Integrity of the Blood-Brain Barrier in Mice

In this Example, the effects of the administration of epinephrine on the integrity of the blood-brain barrier in mice was evaluated. Radiolabelled albumin is the traditional standard to be administered and monitored in order to test the integrity of the blood-brain barrier (Davson. H. (1967) *Physiology of the Cerebrospinal Fluid*, pp. 82-103, J. & A. Churchill, London).

As described above, mice were anaesthetized with ethyl carbamate then given an i.v. injection of either a solution containing radiolabelled leptin (^{125}I, 1.54 x 10^6 cpm) and albumin (^{99}Tc, 3.4 x 10^6 cpm) (labeled solution) in lactated Ringer's solution with 1% bovine serum albumin in 200 µl or the labeled solution plus epinephrine (33 µg). All mice were decapitated with their blood and testis collected after 10 minutes following the leptin injection. The brain (except pituitary and pineals) was removed and counted in a gamma counter (Micromedic 4/200, Horsham, PA) for 3 minutes. Blood was also collected from a cut in the right carotid artery, centrifuged at 2000 g for 10 min. at 4°C, then 50 µl was counted in a gamma counter. Brain/blood and testis/blood ratios were expressed as counts/min./g of brain or testis over counts/min./µl of arterial blood.

Table 3. Co-administration of Epinephrine with Leptin and Albumin

Time post epinephrine administration	brain/blood. (cpm/g) (cpm/µl)	brain/blood (cpm/g) (cpm/µl)	testis/blood (cpm/g) (cpm/µl)	testis/blood (cpm/g) (cpm/µl)
control (no epinephrine)	^{125}I-leptin	^{99}Tc-albumin	^{125}I-leptin	^{99}Tc-albumin
1 min.	12.57	10.02	8.03	4.20
2 min.	15.66	10.22	7.71	4.52
3 min.	16.80	9.87	10.64	4.45
4 min.	22.18	10.14	23.76	8.23
5 min.	22.84	10.85	23.92	8.85
7.5 min.	23.46	10.38	29.55	9.06

Time post epinephrine administration	brain/blood. (cpm/g) (cpm/μl)	brain/blood (cpm/g) (cpm/μl)	testis/blood (cpm/g) (cpm/μl)	testis/blood (cpm/g) (cpm/μl)
10 min.	27.48	11.60	39.02	10.74
12.5 min.	24.36	10.47	44.36	14.42
+ 40 nM epinephrine				
1 min.	19.50	10.38	5.37	2.90
2 min.	22.11	11.28	9.55	4.70
3 min.	27.51	10.84	9.98	4.09
4 min.	36.82	10.46	18.42	6.56
5 min.	28.97	10.62	29.72	7.78
7.5 min.	36.55	10.27	58.68	17.52
10 min.	38.38	11.82	25.38	8.42
12.5 min.	50.53	10.54	152.38	34.09

The results of this experiment indicate that the co-administration of epinephrine with leptin induced an enhanced uptake of leptin by the brain in mice. The data from the time points of 1 min., 2 min., 3 min., 4 min., 5 min., 7.5 min., 10 min., and 12 min. illustrate that the effects of epinephrine on leptin uptake by the brain increase with time as shown in this 12 minute experiment. The co-administration of epinephrine did not enhance the uptake of albumin in the brain of mice. This shows that the increased uptake of leptin by the brain when epinephrine is administered is not the result of a damaged blood-brain barrier, as the amount of ^{99}Tc-albumin crossing the blood-brain barrier remains nearly the same in the presence or absence of epinephrine. In other studies, epinephrine was shown to increase the uptake of both leptin and albumin by testis. While those data indicate that epinephrine may act by disrupting the blood testis barrier, they nonetheless provide evidence that leptin uptake in tissues other than the brain may be enhanced using the cytokines, peptides, neurotransmitters and other molecules according to the present invention.

EXAMPLE 4

Effects of Administration of Amino Acids on Transport of Leptin Across the Blood-Brain Barrier in Mice

In this Example, the effect of administration of various amino acids on transport of leptin across the blood-brain barrier was studied.

As in the prior Examples, mice were anaesthetized with ethyl carbamate. The mice were then given an i.v. injection of either a labeled solution containing radiolabelled human leptin (^{125}I, 1.58 x 10^6 cpm) in lactated Ringer's solution with 1% bovine serum albumin in 200 µl or the labeled solution plus one of the following amino acids (tyrosine or phenylalanine, 10 µg). Blood and brain samples were collected as described in the previous Examples at the following time points post leptin injection (1 min., 2 min., 3 min., 4 min., 5 min., 7.5 min., 10 min., 12.5 min. and 15 min.).

Table 4. Co-administration of Amino Acids with Leptin

Time post amino acid administration	brain/blood (cpm/g)/(cpm/µl)
control (no amino acid)	^{125}I-leptin
1 min.	12.98
2 min.	16.34
3 min.	15.64
4 min.	16.71
5 min.	18.38
7.5 min.	17.42
10 min.	19.00
12.5 min.	22.83
15 min.	26.33
+ tyrosine	
1 min.	13.34
2 min.	14.67

Time post amino acid administration	brain/blood (cpm/g)/(cpm/μl)
3 min.	15.04
4 min.	16.35
5 min.	17.55
7.5 min.	18.26
10 min.	22.90
12.5 min.	24.11
15 min.	23.86
+ phenylalanine	
1 min.	12.24
4 min.	16.15
5 min.	14.39
10 min.	16.67
12.5 min.	20.64
15 min.	23.55

The results of this experiment indicate that the co-administration of tyrosine with leptin enhanced the uptake of leptin by the brain. However, phenylalanine had no such effect. The enhancement of leptin uptake by tyrosine was time dependent over the tested interval of 15 minutes.

EXAMPLE 5

Effects of Administration of Other Amino Acids on Transport of Leptin Across the Blood-Brain Barrier in Mice

In this Example, the effect of other amino acids on the transport of leptin across the blood-brain barrier was studied.

As in the prior Examples, mice were anaesthetized with ethyl carbamate. The mice were then given an i.v. injection of either a solution containing radiolabelled leptin (^{125}I, 1.68×10^6 cpm) in lactated Ringer's solution with 1% bovine serum albumin in 200 μl or the solution plus one of the following amino acids

(arginine, phenylalanine, tryptophan, or tyrosine, 1 mg). Blood and brain samples were collected as described in the previous Examples at 10 min post leptin injection.

Table 5. Co-administration of Amino Acids with Leptin

Time post leptin administration (10 min.)	^{125}I-leptin, brain/blood ± std. dev. (cpm/g)/(cpm/μl)
control (no epinephrine)	15.30 ± 5.05
+ arginine	18.82 ± 2.58
+ phenylalanine	19.83 ± 5.29
+ tryptophan	17.97 ± 4.33
+ tyrosine	23.73 ± 8.84

The results of this experiment indicate that the co-administration of arginine, phenylalanine, or tryptophan with leptin did not affect the uptake of leptin by the brain. However, the administration of tyrosine significantly enhanced leptin uptake by the brain. Similar studies also showed that neither leucine, threonine, nor glycine had an effect on transport of leptin across the blood-brain barrier.

EXAMPLE 6

Effects of Administration of Neurotransmitters on Transport of Leptin Across the Blood-Brain Barrier in Mice

In this Example the effect of certain neurotransmitters on the transport of leptin across the blood-brain barrier was studied.

As described above, groups of mice were anaesthetized with ethyl carbamate. The mice were then given by intracerebroventricular (icv) injection of a solution containing a neurotransmitter such as: acetylcholine, 98 μg; dopamine, 103 μg; epinephrine, 55 μg; histamine, 117 μg; or serotonin, 130 μg. Ten minutes later the mice were given an i.v. injection containing radiolabelled leptin (^{125}I, 1.77 x 10^6 cpm) in lactated Ringer's solution with 1% bovine serum albumin in 100 μl. Blood and brain samples were collected as described in the previous Examples at 10 min post leptin injection.

Table 6. Administration of Neurotransmitters with Leptin

Time post leptin administration (10 min.)	^{125}I-leptin, brain/blood ± std. dev. (cpm/g)/(cpm/μl)
control (no epinephrine)	12.95 ±3.31
+ acetylcholine	13.78 ± 4.73
+ dopamine	13.17 ±2.94
+ epinephrine	15.37 ± 3.12
+ histamine	10.85 ± 4.22
+ serotonin	11.32 ± 3.38

The results of this experiment indicate that the icv administration of the neurotransmitters acetylcholine, dopamine, histamine, and serotonin with leptin had no effect on the uptake of leptin by the brain. The administration of epinephrine by the icv route prior to leptin also did not enhance the uptake of leptin by the brain. This shows that the site at which epinephrine acts to modify leptin transport is on the blood side of the blood-brain barrier.

In another series of studies, neurotransmitters were injected intravenously with 200 nmol/mouse of either acetylcholine, dopamine, epinephrine, histamine, or serotonin. The results of this study indicate that only epinephrine was capable of enhancing transport of leptin across the blood-brain barrier.

EXAMPLE 7

Effects of Administration of Adrenoreceptor Agonists on Transport of Leptin Across the Blood-Brain Barrier in Mice

In this Example, the effect of certain adrenoreceptor agonists on the transport of leptin across the blood-brain barrier was studied.

As in the prior Examples, mice were anaesthetized with ethyl carbamate. The mice were then given an i.v. injection of either a labeled solution containing radiolabelled leptin (^{125}I, 1.98 x 10^6 cpm) in lactated Ringer's solution with 1% bovine serum albumin in 100 μl or the labeled solution plus one of the following agonists: isoproterenol, 25.30 μg; clonidine, 18.66 μg; epinephrine, 14.26 μg; L-

phenylephrine, 14.26 µg; or arterenol, 22.35 µg). Blood and brain samples were collected as described above at 10 min post leptin injection.

Table 7. Co-administration of Adrenoreceptor Agonists with Leptin

Time post administration (10 min.)	^{125}I-leptin, brain/blood ± std. dev. (cpm/g)/(cpm/µl)
control (no agonist)	14.07 ± 1.88
+ isoproterenol	20.61 ± 4.05
+ clonidine	15.05 ± 1.54
+ arterenol (norepinephrine)	23.78 ± 6.35
+ L-phenylephrine	19.19 ± 4.57

The results of this experiment indicate that the co-administration of the adrenoreceptor agonists, isoproterenol and arterenol with leptin enhanced the uptake of leptin by the brain. However, clonidine and L-phenylephrine had no effect on leptin transport

EXAMPLE 8

Effects of Administration of Adrenergic Antagonists on Transport of Leptin Across the Blood-Brain Barrier in Mice

In this Example, the effect of the co-administration of epinephrine with adrenoreceptor antagonists on transport of leptin across the blood-brain barrier was studied.

As described above, mice were anaesthetized with ethyl carbamate. The mice were then given an i.v. injection of either a labeled solution containing radiolabelled leptin (^{125}I, 1.48 x 10^6 cpm) in lactated Ringer's solution with 1% bovine serum albumin and epinephrine (3.33 µg) in 100 µl, or labeled leptin and epinephrine plus one of the following antagonists (phentolamine, 528.4 µg; D,L-propanolol, 4.41 µg; yohimbine, 547.3 µg; or prazosin, saturated solution of 587.8 µg). Blood and brain samples were collected as described above at 10 min post leptin injection.

Table 8. Co-administration of Adrenoreceptor Antagonists and Epinephrine with Leptin

Time post epinephrine administration (10 min.)	^{125}I-leptin, brain/blood ± std. dev. (cpm/g)/(cpm/μl)
control (epinephrine alone)	26.78 ± 4.62
+ phentolamine	18.86 ± 4.27
+ D, L-propanolol	25.68 ± 4.02
+ yohimbine	17.38 ± 3.30
+ prazosin	18.06 ± 1.89

The results of this experiment indicate that the co-administration of the adrenoreceptor antagonists plus epinephrine either had no effect or reduced the uptake of leptin by the brain. Specifically, D,L-propanolol (a β antagonist) had no effect while phentolamine, yohimbine, and prasozin (α antagonists) had a negative effect as compared to the control.

EXAMPLE 9

Effects of Administration of Adrenergic Agonists/Antagonists on Transport of Leptin Across the Blood-Brain Barrier in Mice

In this Example, the effect of adrenoreceptor agonists and antagonists on the transport of leptin across the blood-brain barrier was studied.

As in the prior Examples, mice were anaesthetized with ethyl carbamate. The mice were then given an i.v. injection of either a solution containing radiolabelled leptin (^{125}I, 1.2 x 10^6 cpm) in lactated Ringer's solution with 1% bovine serum albumin in 100 μl or the labeled solution plus one of the following agonists (cirazoline hydrochloride, 25 μg; albuterol, 50 μg; UK 14304; epinephrine, 13.3 μg) or antagonists (benoxathian hydrochloride, 250 μg + epinephrine, 13.3 μg; CGP-12177A (250 μg) and epinephrine, 13.3 μg). Blood and brain samples were collected as described in the previous Examples at 10 min post leptin injection.

Table 9. Co-administration of α_1, α_2 or β Adrenoreceptor Agonists/Antagonists with Leptin

Time post epinephrine administration (10 min.)	^{125}I-leptin, brain/blood ± std. dev. (cpm/g)/(cpm/μl)
control (no epinephrine)	17.0 ± 2.74
+ DMSO	25.1 ± 2.13
+ epinephrine	47.1 ± 17.3
+ cirazoline (α_1 agonist)	45.5 ± 11.3
+DMSO + UK14304 (α_2 agonist)	14.7 ± 3.98
+ albuterol (β agonist)	18.4 ± 2.39
+ epinephrine + CGP 12177A (β antagonist)	39.7 ± 8.70
+ epinephrine + benoxathian (α_1 antagonist)	19.7 ± 5.01

The results of this experiment indicate that the co-administration of the α_1 agonist cirazoline with leptin enhanced the uptake of leptin by the brain. The data also show that the α_1 antagonist benoxathian blocked the enhancing effect of epinephrine.

EXAMPLE 10

Modulating Leptin Transport by Tumor Necrosis Factor α (TNF-α)

TNF-α (cachexin) is a cytokine about the same size as leptin that is transported across the blood-brain barrier and also has effects on feeding. This suggests the possibility that TNF may modulate the transport of leptin across the blood-brain barrier. Similarly, leptin may play a role in the transport of TNF across the blood-brain barrier. This hypothesis was tested using two experimental paradigms, an acute model, and a chronic model.

Acute

This experiment determined whether the acute administration of TNF would acutely affect the entry of radioactively labeled leptin (I-leptin) into the brain.

Three groups of mice were tested. One group received labeled leptin (described in Examples set out above) alone; a second group received labeled leptin plus 1 μg/mouse of mouse TNF; a third group received 1 μg /mouse of human TNF and labeled leptin. After injection, brain and blood samples were obtained at times similar to those used in the Examples set out above over time. (It should be noted that this is a high dose of TNF.) There was no difference among these groups in the amount of labeled leptin transported into the mouse brain. These data show that TNF and leptin do not share the same transporter (no competition or inhibition of leptin transport) nor does it acutely upregulate the leptin transporter (no enhancement of leptin transport).

Chronic

This experiment was performed in mice that were genetically altered so that both of the receptors for TNF were "knocked out" and therefore did not express active TNF receptors. As such, these mice were insensitive to TNF. The rate of uptake of labeled leptin in these mice was then determined in comparison to controls. The amount of unlabeled leptin that was needed to inhibit the leptin transporter in these mice was also determined.

The entry rate of 0.477 μl/g-min for labeled leptin is similar to that typically found in normal mice. However, the entry of I-leptin into the brain was not inhibited by 0.1 or 0.3 μg/mouse of unlabeled leptin and 1.0 μg/mouse inhibited the entry rate by only 40%. In normal mice, 0.3 μg inhibits entry by 50% and 1 μg/mouse inhibits entry by 95%.

These data suggest that TNF receptor knockout mice have an altered transporter for leptin across the blood-brain barrier. Chronic TNF exposure, perhaps especially during development, is likely needed for the normal functioning of the leptin transporter. This, therefore, represents another class of compounds (cytokines), in addition to the adrenergic agonist, amino acids, and other compositions described above that can modulate the transport of leptin across the BBB.

EXAMPLE 11

Modulation of Leptin Uptake by Adenosine and Glutamate

Studies were conducted to determine whether compounds which interact with purinergic receptors or with glutamate (glutaminergic) receptors are capable of modulating the uptake of leptin into the brain.

In particular, these studies were conducted to determine whether the acute administration of adenosine, arginine, or glutamate could affect the entry of radioactively labeled leptin (I-leptin) into the brain. Four groups of mice were tested. One group received labeled leptin (as described in examples set out above) alone, a second group received labeled leptin plus adenosine (0.4 mmol/kg), a third group received labeled leptin plus arginine (10 mg/mouse), and a fourth group received labeled leptin plus glutamate at the dosage of 10 mg/mouse.

L-Arginine is an essential amino acid included below as a control. After injection, brain and blood samples were obtained at 10 min post leptin injection as described. The brain/serum ratios have been corrected for vascular space by subtracting 10 µl/g.

Table 10. Co-administration of Adenosine, Arginine, or Glutamate with Leptin

Compound	^{125}I-leptin,, brain/blood ± std dev. (cpm/g) (cpm/µl)
I-leptin Only (Control)	21.6 ± 1.96
Adenosine	14.9 ± 4.79
Arginine	21.8 ± 5.29
Glutamate	9.67 ± 2.3

The results of this experiment shown in Table 10 indicate that co-administration of the purinergic agonist adenosine or the glutamate agonist significantly decreased the transport of leptin into the brain. Co-administration of arginines an essential amino acid had no effect on leptin transport.

The results set out above show that purinergic agonists such as adenosine are useful in decreasing uptake of leptin into the brain and thus may act as

therapeutic agents in pathological conditions or under other circumstances wherein a decrease in leptin uptake in the brain is desired.

Similarly the results shown in Table 10 show that agonists (*e.g.*, glutamate) that interact with glutamate receptors including the ionotropic receptors (*e.g.* AMPA, and N-methyl-D-aspartate receptors) and metabotropic receptors may also be useful in the same context as the purinergic agonists described above.

While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those skilled in the art. All of the foregoing references are hereby incorporated by reference.

CLAIMS

I CLAIM:

1. A method for modulating the transport of leptin across the blood-brain barrier of a mammal, the method comprising:

administering to the mammal an effective amount of one or more compositions selected from the group consisting of adrenergic agonists, adrenergic antagonists, neurotransmitters, cytokines, amino acids, opiate peptides, purinergic agonists, glutaminergic agonists and metabolites thereof.

2. The method of claim 1 wherein said one or more compositions is administered to the mammal via a route of administration selected from the group consisting of intravenous, intraarterial, intramuscular, intraperitoneal, subcutaneous, topical, intraocular, intracerebroventricular, intracisternal, intrathecal, intradermal, transdermal, nasal, oral and pulmonary.

3. The method of claim 1 further comprising co-administering to the mammal a leptin selected from the group of leptins comprising the amino acid sequence set out as SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, consensus leptins, variants, analogs, leptin fusion proteins, chemically modified derivatives of leptin, and fragments thereof, said leptin optionally having an N-terminal methionine.

4. The method of claim 2 further comprising co-administering to the mammal a leptin selected from the group of leptins comprising the amino acid sequence set out as SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, consensus leptins, variants, analogs, leptin fusion proteins, chemically modified derivatives of leptin, and fragments thereof, said leptin optionally having an N-terminal methionine.

5. The method of claims 1, 2, 3, or 4 wherein the one or more adrenergic agonists are selected from the group consisting of epinephrine, isoproterenol, arterenol, and cirazoline.

6. The method of claim 1, 2, 3, or 4 wherein the one or more adrenergic antagonists are selected from the group consisting of yohimbine, phentolamine, prasozin, and benoxathian.

7. The method of any one of claims 1, 2, 3, or 4 wherein the cytokine is TNF-α.

8. The method of claim 1, 2, 3, or 4 wherein the amino acid is tyrosine.

9. The method of claim 1, 2, 3, or 4 wherein the purinergic agonist is adenosine.

10. The method of claim 1, 2, 3, or 4 wherein the glutaminergic agonist is glutamate.

11. A method for modulating body weight in a mammal, the method comprising:

administering to the mammal an effective amount of one or more compositions selected from the group consisting of adrenergic agonists, adrenergic antagonists, neurotransmitters, cytokines, amino acids, opiate peptides, purinergic agonists, glutaminergic agonist, and metabolites thereof.

12. The method of claim 11 wherein said one or more compositions is administered to the mammal via a route of administration selected from the group consisting of intravenous, intraarterial, intramuscular, intraperitoneal, subcutaneous,

topical, intraocular, intracerebroventricular, intracisternal, intrathecal, intradermal, topical transdermal, nasal, oral and pulmonary.

13. The method of claim 11 further comprising co-administering to the mammal of a leptin selected from the group of leptins comprising the amino acid sequence set out as ID No. 2, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, consensus leptins, variants, analogs, leptin fusion proteins, chemically modified derivatives of leptin, and fragments thereof, said leptin optionally having an N-terminal methionine.

14. The method of claim 12 further comprising co-administering to the mammal a leptin selected from the group of leptins comprising the amino acid sequence set out as SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, consensus leptins, variants, analogs, leptin fusion proteins, chemically modified derivatives of leptin, and fragments thereof, said leptin optionally having an N-terminal methionine.

15. The method of claim 11 wherein modulating body weight is decreasing body weight.

16. The method of claims 11, 12, 13, 14, or 15 wherein the one or more adrenergic agonists are selected from the group consisting of epinephrine, isoproterenol, arterenol, and cirazoline.

17. The method of claims 11, 12, 13, 14, or 15 wherein the amino acid is tyrosine.

18. The method of claims 11, 12, 13, or 14 wherein the cytokine is TNF-α.

19. The method of claim 11 wherein modulating body weight is increasing body weight.

20. The method of claims 11, 12, or 19 wherein the one or more adrenergic antagonists are selected from the group consisting of yohimbine, phentolamine, prasozin, and benoxathian.

21. The method of claim 11, 12, or 19 wherein the purinergic agonist is adenosine.

22. The method of claim 11, 12, or 19 wherein the glutaminergic agonist is glutamate.

23. A method for modulating appetite in a mammal, the method comprising:

administering to the mammal an effective amount of one or more compositions selected from the group consisting of adrenergic agonists, adrenergic antagonists, neurotransmitters, cytokines, amino acids, opiate peptides, purinergic agonists, glutaminergic agonists, and metabolites thereof.

24. The method of claim 23 wherein said one or more compositions is administered to the mammal via a route of administration selected from the group consisting of intravenous, intraarterial, intramuscular, intraperitoneal, subcutaneous, topical, intraocular, intracerebroventricular, intracisternal, intrathecal, intradermal, topical, transdermal, subcutaneous, nasal, oral, and pulmonary.

25. The method of claim 23 further comprising co-administering to the mammal a leptin selected from the group of leptins comprising the amino acid sequence set out as SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, consensus leptins, variants, analogs, leptin fusion proteins, chemically modified derivatives of leptin, and fragments thereof, said leptin optionally having an N-terminal methionine.

26. The method of claim 24 further comprising the co-administering to the mammal a leptin selected from the group of leptins comprising the amino acid sequence set out as SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, consensus leptins, variants, analogs, leptin fusion proteins, chemically modified derivatives of leptin, and fragments thereof, said leptins optionally having an N-terminal methionine.

27. The method of claims 23, 24, 25, or 26 wherein the one or more adrenergic agonists are selected from the group consisting of epinephrine, isoproterenol, arterenol, and cirazoline.

28. The method of claims 23, 24, 25, or 26 wherein the one or more adrenergic antagonists are selected from the group consisting of yohimbine, phentolamine, prasozin, and benoxathian.

29. The method of claims 23, 24, 25, or 26 wherein the cytokine is TNF-α.

30. The method of claims 23, 24, 25, or 26 wherein the amino acid is tyrosine.

31. The method of claims 23, 24, 25, or 26 wherein said purinergic agonist is adenosine.

32. The method of claims 23, 24, 25, or 26 wherein the glutaminergic agonist is glutamate.

33. A pharmaceutical composition useful for modulating body weight, the composition comprising a leptin selected from the group of leptins comprising the amino acid sequence set out in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, consensus leptins, variants, analogs, leptin fusion proteins,

chemically modified derivatives of leptin, and fragments thereof, said leptin optionally having an N-terminal methionine, in combination with an effective amount of an andrenergic agonist, adrenergic antagonist, neurotransmitter, cytokine, amino acid, opiate peptide, purinergic agonist, glutaminergic agonist, and metabolites thereof.

34. The pharmaceutical composition of claim 33 wherein the one or more adrenergic agonists are selected from the group consisting of epinephrine, isoproterenol, arterenol, and cirazoline.

35. The pharmaceutical composition of claim 33 wherein the one or more adrenergic antagonists are selected from the group consisting of yohimbine, phentolamine, prasozin, and benoxathian.

36. The pharmaceutical composition of claim 33 wherein the cytokine is TNF-α.

37. The pharmaceutical composition of claim 33 wherein the amino acid is tyrosine.

38. The pharmaceutical composition of claim 33 wherein said purinergic agonist is adenosine.

39. The pharmaceutical composition of claim 33 wherein the glutaminergic agonist is glutamate.

40. The use of one or more adrenergic agonists or metabolites thereof for the manufacture of a medicament for modulating the transport of leptin across the blood-brain barrier.

41. The use of claim 40 wherein the one or more adrenergic agonists are selected from the group consisting of epinephrine, isoproterenol, arterenol, and cirazoline.

42. The use of one or more adrenergic antagonists or metabolites thereof for the manufacture of a medicament for modulating the transport of leptin across the blood-brain barrier.

43. The use of claim 42 wherein the one or more adrenergic antagonists are selected from the group consisting of yohimbine, phentolamine, prasozin, and benoxathian.

44. The use of one or more neurotransmitters or metabolites thereof for the manufacture of a medicament for modulating the transport of leptin across the blood-brain barrier.

45. The use of one or more peptide hormones for the manufacture of a medicament for modulating the transport of leptin across the blood-brain barrier.

46. The use of one or more cytokines for the manufacture of a medicament for modulating the transport of leptin across the blood-brain barrier.

47. The use of claim 46 wherein the cytokine is TNF-α.

48. The use of one or more amino acids for the manufacture of a medicament for modulating the transport of leptin across the blood-brain barrier.

49. The use of claim 48 wherein the amino acid is tyrosine.

50. The use of one or more opiate peptides for the manufacture of a medicament for modulating the transport of leptin across the blood-brain barrier.

51. The use of one or more purinergic agonists for the manufacture of a medicament for modulating the transport of leptin across the blood-brain barrier.

52. The use of claim 51 wherein said purinergic agonist is adenosine.

53. The use of a glutaminergic agonist for the manufacture of a medicament for modulating the transport of leptin across the blood-brain barrier.

54. The use of claim 53 wherein the glutaminergic agonist is glutamate.

55. The uses according to any one of claims 38 to 54 further comprising the use of leptin for the manufacture of said medicament for modulating the transport of leptin across the blood-brain barrier.

56. The use according to claim 55 wherein said leptin is selected from the group consisting of leptin comprising the amino acid sequence set out as SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, consensus leptins, variants, analogs, leptin fusion proteins, chemically modified derivatives of leptin, and fragment thereof, said leptin optionally having an N-terminal methionine.

57. The use of one or more adrenergic agonists or metabolites thereof for the manufacture of a medicament for modulating the body weight of a mammal.

58. The use of claim 55 wherein the one or more adrenergic agonists are selected from the group consisting of epinephrine, isoproterenol, arterenol, and cirazoline.

59. The use of one or more adrenergic antagonist or metabolites thereof for the manufacture of a medicament for modulating the body weight of a mammal.

60. The use of claim 59 wherein the one or more adrenergic antagonists are selected from the group consisting of yohimbine, phentolamine, prasozin, and benoxthian.

61. The use of one or more neurotransmitters or metabolites thereof for the manufacture of a medicament for modulating the body weight of a mammal.

62. The use of one or more peptide hormones for the manufacture of a medicament for modulating the body weight of a mammal.

63. The use of one or more cytokines for the manufacture of a medicament for modulating the body weight of a mammal.

64. The use of claim 63 wherein the cytokine is TNF.

65. The use of one or more amino acids for the manufacture of a medicament for modulating the body weight of a mammal.

66. The use of claim 65 wherein the amino acid is tyrosine.

67. The use of one or more opiate peptides for the manufacture of a medicament for modulating the body weight of a mammal.

68. The use of one or more purinergic agonists for the manufacture of a medicament for modulating the body weight of a mammal.

69. The use of claim 68 wherein said purinergic agonist is adenosine.

70. The use of a glutaminergic agonist for the manufacture of a medicament for modulating the body weight of a mammal.

71. The use of claim 70 wherein the glutaminergic agonist is glutamate.

72. The uses according to any one of claims 57, 58, 65, or 66 further comprising the use of leptin for the manufacture of said medicament for modulating the body weight of a mammal.

73. The use according to claim 72 wherein said leptin is selected from the group consisting of leptin comprising the amino acid sequence set out as SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, consensus leptins, variants, analogs, leptin fusion proteins, chemically modified derivatives of leptin, and fragments thereof, said leptin optionally having an N-terminal methionine.

74. The use of any one of claims 57, 58, 65, 66, or 72 wherein modulating body weight is reducing body weight.

75. The use according to claim 72 wherein modulating body weight is reducing body weight.

76. The use of any one of claims 57, 59, 60, 68, 69, 70, or 71 wherein modulating body weight is increasing body weight.

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Ser Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro
             55                  60                  65

atc ctg acc tta tcc aag atg gac cag aca ctg gca gtc tac caa cag   297

Ile Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln
         70                  75                  80

atc ctc acc agt atg cct tcc aga aac gtg atc caa ata tcc aac gac   345
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Ile Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln Ile Ser Asn Asp
     85                  90                  95

ctg gag aac ctc cgg gat ctt ctt cac gtg ctg gcc ttc tct aag agc   393

Leu Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser
100                 105                 110                 115

tgc cac ttg ccc tgg gcc agt ggc ctg gag acc ttg gac agc ctg ggg   441

Cys His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly
                120                 125                 130

ggt gtc ctg gaa gct tca ggc tac tcc aca gag gtg gtg gcc ctg agc   489

Gly Val Leu Glu Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser
            135                 140                 145

agg ctg cag ggg tct ctg cag gac atg ctg tgg cag ctg gac ctc agc   537

Arg Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser
        150                 155                 160

cct ggg tgc tgaggccttg aaggtcactc ttcctgcaag gactnacgtt           586

Pro Gly Cys
    165
aagggaagga actctggttt ccaggtatct ccaggattga agagcattgc atggacaccc 646

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<212> PRT
<213> Homo sapiens

<400> 4

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Phe Tyr Val Gln Ala Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys
                 20                  25                  30

Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr
             35                  40                  45

Gln Ser Val Ser Ser Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro
         50                  55                  60

Gly Leu His Pro Ile Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala
     65                  70                  75

Val Tyr Gln Gln Ile Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln
 80                  85                  90                  95

Ile Ser Asn Asp Leu Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala
                100                 105                 110
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Phe Ser Lys Ser Cys His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu
115 120 125

Asp Ser Leu Gly Gly Val Leu Glu Ala Ser Gly Tyr Ser Thr Glu Val
130 135 140

Val Ala Leu Ser Arg Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln
145 150 155

Leu Asp Leu Ser Pro Gly Cys
160 165

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<211> 146
<212> PRT
<213> Mus musculus

<220>
<223> Mature mouse ob (leptin)

<400> 5
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1 5 10 15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Gln Ser Val Ser Ala
20 25 30

Lys Gln Arg Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
35 40 45

Leu Ser Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln Val
50 55 60

Leu Thr Ser Leu Pro Ser Gln Asn Val Leu Gln Ile Ala Asn Asp Leu
65 70 75 80

Glu Asn Leu Arg Asp Leu Leu His Leu Leu Ala Phe Ser Lys Ser Cys
85 90 95

Ser Leu Pro Gln Thr Ser Gly Leu Gln Lys Pro Glu Ser Leu Asp Gly
100 105 110

Val Leu Glu Ala Ser Leu Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
115 120 125

Leu Gln Gly Ser Leu Gln Asp Ile Leu Gln Gln Leu Asp Val Ser Pro
130 135 140

Glu Cys
145

<210> 6
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<213> Homo sapiens

<220>
<223> Mature human ob (leptin)

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Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
 1               5                  10                  15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Gln Ser Val Ser Ser
            20                  25                  30

Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
        35                  40                  45

Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
    50                  55                  60

Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln Ile Ser Asn Asp Leu
65                  70                  75                  80

Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys
                85                  90                  95

His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly
            100                 105                 110

Val Leu Glu Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
        115                 120                 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro
    130                 135                 140

Gly Cys
145
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DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **"Modulation of the Blood-Brain Barrier Transporter for Leptin,"** the specification of which was filed on August 23, 1999 as Application Serial No. 60/150,300. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

			Priority Claimed	
(Application Serial Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No
(Application Serial Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

(Application Serial Number) (Day/Month/Year Filed)

(Application Serial Number) (Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)
(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

APPLICABLE RULES AND STATUTES

37 CFR 1:56. DUTY OF DISCLOSURE - INFORMATION MATERIAL TO PATENTABILITY (Applicable Portion)

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and
(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentability defines, to make sure that any material information contained therein is disclosed to the Office.

Information relating to the following factual situations enumerated in 35 USC 102 and 103 may be considered material under 37 CFR 1.56(a).

35 U.S.C. 102. CONDITIONS FOR PATENTABILITY: NOVELTY AND LOSS OF RIGHT TO PATENT

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent, or

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States, or

(c) he has abandoned the invention, or

(d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate filed more than twelve months before the filing of the application in the United States, or

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraph (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or

(f) he did not himself invent the subject matter sought to be patented, or

(g) before the applicant's invention thereof the invention was made in this country by another who had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other.

35 U.S.C. 103. CONDITIONS FOR PATENTABILITY; NON-OBVIOUS SUBJECT MATTER (Applicable Portion)

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

35 U.S.C. 112. SPECIFICATION (Applicable Portion)

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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Nate F. Scarpelli (22,320)	James P Zeller (28,491)	Robert M. Gerstein (34,824)	Gregory C. Mayer (38,238)
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State or Country	State or Country
Date ☒	Signature ☒

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City (Zip)	City (Zip)
State or Country	State or Country
Date ☒	Signature ☒

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City (Zip)	City (Zip)
State or Country	State or Country
Date ☒	Signature ☒